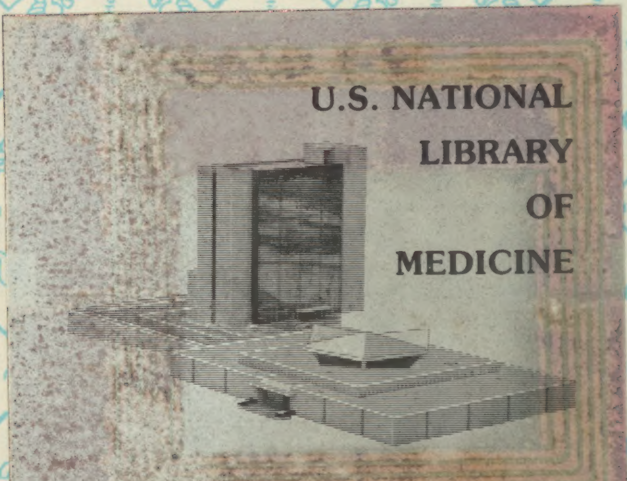
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A Laboratory Outline of Embryology

With Special Reference to the Chick and the Pig

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A Laboratory Outline of Embryology

With Special Reference to the Chick and the Pig

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PREFACE

The original publication of the *Embryology of the Chick and the Pig*, with its revision (Lillie, 1904 and 1906), has served for the study of embryology for medical students in many schools since that time; practical experience in the meantime has proved to the writers that a course in embryology for medical students can be presented more effectively by studying the development of each organ system separately than by consideration of the entire embryo at time intervals.

These outlines, arranged by the junior writer, are the result of several revisions of the original draft, and have been employed in the medical course during the past six years at the University of Chicago. It is hoped that they may prove useful in other institutions as an indication of a standard type of laboratory study for medical students. With proper additions, by an instructor, the outlines may be adapted to a more thorough laboratory course than is considered necessary for the medical student.

Specific directions have been included to enable the student to locate certain regions and structures; it is assumed that the sections are cut transversely

to the longest axis of the embryo, and that sections of approximately the following thickness are used: 33-hour chick 10 μ-15 μ; 48-hour chick, 15 μ; 72-hour chick, 15 μ; 10-mm. pig, 15 μ.

References to figures and pages refer to the *Development of the Chick* (Lillie, 2d ed.), *Textbook of Embryology* (Prentiss and Arey, 3d ed.), and the *Development of the Human Body* (McMurrich, 6th ed.), all of which the student is advised to have at hand.

HULL ZOÖLOGICAL LABORATORIES
UNIVERSITY OF CHICAGO
1923

TABLE OF CONTENTS

PROVISIONAL TIME SCHEDULE

I. Morphology and gross anatomy	
Chick embryo, 33 hours, living and entire preparation	$2\frac{1}{2}$ hrs.
Chick embryo, 33 hours, sections	$3\frac{1}{2}$ hrs.
Chick embryo, 48 hours, living and entire preparation	3 hrs.
Chick embryo, 72 hours, living and entire preparation	3 hrs.
Chick embryo, five days	1 hr.
Pig embryo, 10-mm. dissection	2 hrs.
Pig embryos, 15-mm. and 25-mm. dissection	3 hrs.
II. Embryonic membranes, coelome, mesenteries, and placenta	
Chick embryos, 33 hours and 48 hours	3 hrs.
Chick embryo, 72 hours	3 hrs.
Pig embryo, 10 mm.	3 hrs.
Placenta of pig	3 hrs.
III. Ectodermal derivatives	
Nervous system of chicks and pig	3 hrs.
Organs of special sense, of chicks and pig	3 hrs.
IV. Entodermal derivatives	
Chick embryos, 33 hours, 48 hours and 72 hours	3 hrs.
Pig embryo 10 mm.	3 hrs.
V. Mesodermal derivatives	
Urinogenital system of chicks and pig	3 hrs.
Heart of chicks and pig	3 hrs.
Arterial system of chicks and pig	5 hrs.
Venous system of chicks and pig	6 hrs.
Derivatives of a somite	1 hr.
	60 hrs.

METHODS

The methods of study employed in the following outlines are: (1) Study of the living embryo. (2) Study of the entire embryo, (*a*) as an opaque object with the dissecting microscope; (*b*) with the compound microscope after killing, hardening, staining, clearing, and mounting. (3) Study of embryos by dissection, especially in later stages. (4) Study of organs and systems from the serial sections with the microscope.

Lillie's *Development of the Chick* and Prentiss and Arey's *Textbook of Embryology* should be at hand continually. Notes consist of answers to questions.

STUDY OF THE LIVING EMBRYO OF THE CHICK

This is best performed under warm normal salt solution (0.75 per cent NaCl heated to about 38° C.). Open the egg by gradually picking away the shell at the broad end, making an aperture large enough to permit of the escape of the entire egg contents without breaking the yolk. Invert the open end of the egg *beneath* the salt solution and allow the contents to flow out. The embryo may now be examined on the surface of the yolk, as the blastoderm almost invariably turns up, or may

easily be made to do so. The blastoderm can now be separated by cutting around it *outside the area vasculosa* with fine scissors. It should be gently floated into a Syracuse watch crystal *submerged* in the salt solution. The crystal and its contents may then be lifted out. The vitelline membrane, a delicate *transparent membrane* covering the blastoderm, must next be removed.

I. MORPHOLOGY AND GROSS ANATOMY

A. CHICK EMBRYO WITH FROM TEN TO FOURTEEN SOMITES (TWENTY-NINE TO THIRTY-FOUR HOURS)

1. *The egg.*—While opening the egg, observe that the *shell membrane* is double. The *air chamber* at the broad end is between its two layers. After pouring the egg into the bowl, observe the twisted cords of albumen in the "white" (*chalazae*). They are attached to the denser layer of albumen (secreted by the glands of the oviduct) that closely surrounds the yolk. What function may they have? The *yolk* or *vitellus* is the true *ovum*, the other parts being merely *envelopes*. Observe the *vitelline membrane* surrounding the yolk. (See Lillie, Fig. 32.)

2. *The living embryo.*—How much of the yolk is covered by the *blastoderm* (the living part of the egg)? In the center of the blastoderm is a transparent slipper-shaped area (*area pellucida*), in which

the embryo appears as a narrow opaque streak. The part of the blastoderm external to the pellucid area is known as the *area opaca*. How much of the latter is occupied by the *area vasculosa?* The remainder of the opaque area is known as the *area vitellina*. Make a sketch of the egg and embryo to show these relations.

3. *Entire mount including the vascular area (stained embryo)*.—

a) In the vascular area observe the irregular deeply stained masses (*blood islands*). At this stage it will be found that they are inclosed in wide anastomosing tubes, the *extra-embryonic blood vessels*, that open peripherally into the bounding *sinus terminalis*. How are the extra-embryonic connected with the embryonic blood vessels?

b) The embryo. Make a careful drawing *to scale* (×ca. 25 or 30) of the embryo and *area vasculosa*. State whether your drawing is from the *upper* or from the *under* side. (Lillie, Figs. 51, 61, 65.)

The following structures can be readily identified (others will be seen, but are better understood after study of sections):

(1) The *neural tube* forms the axis of the embryo. In its anterior region identify *forebrain* (*prosencephalon*), *optic vesicles*, *mid-brain* (*mesencephalon*), *hind-brain* (*rhombencephalon*). The secondary subdivisions of the hind-brain are the *neuromeres;*

how many? The portion of the neural tube (*cord*, *myelon*) back of the hind-brain is closed in front, but open behind.

(2) The *auditory pit*, a slight depression of thickened ectoderm lateral to the fourth and fifth neuromeres, *may* be seen in the older embryos of this stage (13 to 14 somites).

(3) The *primitive streak* is inclosed by the diverging folds of the myelon.

(4) The *head fold*. The head of the embryo projects freely above the blastoderm. The fold uniting the ventral surface of the head with the blastoderm is known as the "head fold."

(5) The *mesoblastic somites*, appearing on each side of the neural tube. Number? The series is continued behind by the undivided *segmental plate*.

(6) The *heart* lies beneath the hind-brain in a special portion of the body cavity bounded in front by the head fold and behind by the converging limbs of the *splanchnopleure*. Its posterior (venous) end receives the *omphalomesenteric veins* from the vascular area. Its anterior (arterial) end is prolonged into the *ventral aorta*. Its axis is somewhat bent at this stage. In what direction?

(7) The *proamnion*, a transparent two-layered portion of the blastoderm beneath and lateral and anterior to the head of the embryo.

(8) The *head fold* of the *amnion* is beginning to fold over the anterior end of the head in the older embryos of this stage.

(9) The *fore-gut*, the lateral boundaries of which may be seen between the ectoderm of the head and the brain.

(10) The *notochord*, a dark streak apparently in the center of, but actually ventral to, the brain and neural tube.[1]

4. *Study of transverse sections.*—The embryo has been cut into a series of transverse sections of uniform thickness of 15 micromillimeters (0.015 mm.) The number of any section in the series, therefore, in comparison with the entire number, will enable the student to ascertain its position with relation to the entire embryo. *Each section drawn is to be located in its position by a line across the drawing of the entire embryo.*

Study each section drawn under the high power as well as under the low power. Some drawings of parts of sections should be made with the high power to show the character of the individual cells. For low-power drawings use colors to distinguish ectoderm (blue), mesoderm (red), and entoderm (yellow), and do not draw in cells.

[1] The part of the embryo back to the last somite formed, represents the head and cervical region of the bird. The remainder of the embryo is to be formed from the posterior unsegmented region.

After the segmentation of the ovum the multiplication of cells continues, and these gradually arrange themselves in such a manner as to form three distinct sheets or laminae, which are named germ layers. These layers are designated: the outer as ectoderm, the inner as entoderm, and the middle as mesoderm. The student should familiarize himself with the appearance of these layers and the modification of each in the production of the various organs and systems. (For derivatives of germ layers see Prentiss and Arey, 3d ed., p. 56.)

Make *careful* drawings of transverse sections through the following regions, under the low power of the microscope:

(*a*) Primitive streak.

(*b*) Through region of the open neural tube.

(*c*) Through the sixth or seventh somite of the series.

(*d*) Posterior half of heart.

(*e*) Through mid-brain.

(*f*) Through optic vesicles.

Identify and *label fully*, all the parts to be observed.

a) The section illustrates the undifferentiated germ layers from which the embryo is formed, and the formation of mesoderm from the primitive streak. It represents, in a sense, the youngest part of the embryo, and as development proceeds the primitive streak as such disappears, becoming

part of the embryo proper (see Lillie, chap. iv and Fig. 39). Note (1) that the entoderm (lower layer) is separate, while mesoderm and ectoderm are not separate layers in the median line but merge inseparably in the undifferentiated mass of cells; this is the *primitive streak*. The depression in the primitive streak is the *primitive groove*. (2) Mesoderm appears as wings from the thickened central portion and is split peripherally into two layers known as the somatic (upper) and splanchnic layers; the combination of the somatic layer with the ectoderm is known as the *somatopleure*, that of the splanchnic layer with the entoderm as *splanchnopleure*. (3) The space between these two layers is the *body cavity* (coelome). What is the significance of the primitive streak? (Read Lillie, pp. 69–86.)

b) As one proceeds anteriorly from the primitive streak into an older, differentiated region of the embryo the germ layers appear slightly modified. Note (1) that the *medullary plate* (thickened ectoderm) has folded upward, producing the *neural groove* (bounded by the *neural folds*) which is not yet closed above. (2) The rodlike *notochord* (reckoned as entoderm) lies directly under the neural tube.[1] (3) The somatic and splanchnic layers of

[1] The notochord is the first indication of a skeletal axis and is the center about which mesenchyme (sclerotome) is converted, first into cartilage and finally into bone, to form the centra of vertebrae.

mesoderm are separated within the embryo producing the *coelome*, which is continuous with the extra-embryonic body cavity. Do blood vessels appear in this section, and if so where?

c) This section should be about half-way between the posterior end of the heart and the last somite and through the center of a mesoblastic somite (see Lillie, Fig. 68). The ectodermal folds (neural or medullary folds) have met dorsally in the median line, completing the closed *neural tube*. The *notochord* appears ventral to the neural tube. The mesoderm is much thickened and consists on each side of the median line of the following parts: (1) the *mesoblastic somite*, a block of cells that radiate from a central mass; (2) the *intermediate cell mass* (*nephrotome*) between (1) and (3); (3) the *lateral plate*, split into the somatic and splanchnic layers. Is there a *coelome* in this section? The two large blood vessels immediately beneath the somites are the *dorsal aortae*. Note the numerous blood vessels in the splanchnopleure; do you find any in the somatopleure? The entodermal layer always appears as the ventral boundary of the embryo or blastoderm; in the region of the embryo it folds to produce the gut, and peripherally continues as the lining of the yolk sac. This relation should always be kept in mind.

d) Trace the parts shown in (*c*) forward to this section. The splanchnopleure folds ventrally and

has given rise to two new structures in this region: the *pharynx* or anterior division of the *fore-gut*, produced by the growing together ventrally of the splanchnopleure from the two sides, and the heart, produced in the same manner from splanchnic mesoderm. The *fore-gut* appears as a crescentic cavity with lateral projections. What do these signify? Notice again at the open end of the fore-gut (a few sections posterior to *d*) that the gut is formed by the gradual folding and growing together of the splanchnopleure. The *heart*, formed from the splanchnic layer of mesoderm, is attached by a dorsal mesentery (mesocardium) to the under wall of the pharynx. Note its two layers; *muscular* (*myocardium*) and *endothelial* (*endocardium*) both of which are mesoderm. The cavity in which it is situated (*pericardial*) is an enlarged part of the general body cavity or coelome. Do the dorsal aortae appear in this section? To what level of the embryo do they extend anteriorly?

e) Choose a section through the oral plate, the point of fusion between ventral ectoderm of the head and entoderm of the ventral side of the pharynx. This is the site of the future mouth (see Lillie, Figs. 55, 67). The fore-gut in the region of mid- and hind-brain is known as the pharynx. Identify *dorsal* and *ventral aortae*, *proamnion*, *parietal cavity* (extra-embryonic), *somatopleure*, *splanchnopleure*. Find out, by tracing both for-

ward and backward, if the ventral aortae have any connection with heart or dorsal aortae. Compare the mesoderm of this section with that of section *c;* the loose unorganized mesoderm is known as mesenchyme. Is the embryo connected with the blastoderm in this section?

f) Do you find entodermal structures in this section? Where did the notochord disappear? Draw in the accompanying section of the blastoderm. What is the proamnion? Identify and label parts.

B. CHICK EMBRYO WITH FROM TWENTY-FOUR TO TWENTY-NINE SOMITES (FORTY-FOUR TO FORTY-EIGHT HOURS)

1. *The living embryo.*—Describe carefully the changes visible to the naked eye since the thirty-fourth hour. Observe the beating of the heart and the circulation of the blood. Can the heartbeat after stopping be renewed by slight heating? Make a sketch of the embryo on the egg showing the relation of the various areas of the blastoderm. Remove the embryo with the entire vascular area and preserve it.

2. *Entire mounts.*—The most striking changes concern the region of the head. By more rapid growth of the dorsal surface the head has become bent (*cephalic flexure*) in the region of the midbrain, so that the forebrain and part of the midbrain form almost a right angle with the rest of the

head. Moreover, the head has become so far free from the blastoderm, and so compressed laterally, that it now lies on its side (which side?). The dorsal side of the trunk, on the other hand, is still turned up, so that there is a twisting of the embryo just back of the heart. About the head three layers may be seen: brain wall, ectoderm of head, and amnion. The *tail fold* is not yet formed, or has just begun. (See Lillie, Fig. 93.)

a) Brain: The prosencephalon now exhibits two divisions (*telencephalon* and *diencephalon*) and the rhombencephalon likewise two (*metencephalon* and *myelencephalon*): the mesencephalon is undivided.

b) The *optic vesicles* are relatively smaller in relation to the brain than in the 33-hour chick (not actually smaller, of course). To which division of the forebrain are they attached? Distinguish *inner* and *outer layers* of the *retina*, the *lens* and the *choroid fissure.*

c) The *auditory vesicles* (*otocyst*) are now closed (?) sacs. Above which visceral arch do they lie?

d) The heart has grown greatly in length, and, its two ends being fixed, it has become doubled on itself. Identify *atrium*, *ventricle*, and *bulbus arteriosus*. What is the relation of the heart to the main afferent and efferent blood vessels?

e) Two or three *visceral* (*pharyngeal*) *pouches* are now visible. They may be found ventral to the

region of the myelencephalon. The first or *hyomandibular* pouch is bounded in front by the *first visceral* or *mandibular arch*, and behind by the *second visceral* or *hyoid arch;* the second pouch is bounded in front by the *hyoid*, and behind by the *third visceral arch;* the third pouch is bounded in front by the *third visceral arch* and behind by the *fourth.* (See Lillie, pp. 173–78.) Running through each visceral arch note the small clear blood vessel, the *aortic arches;* why called arches?

f) How many mesoblastic somites are there? What is the condition of the mesoblastic segmental plate?

g) How far back is the fore-gut closed?

h) Find the head fold of the amnion. How far back is the amnion closed?

i) What changes have taken place in the vascular area?

j) Has the tail fold formed? Do you find the primitive streak?

Make a drawing of the entire embryo illustrating all of the foregoing points ($\times 15$ or 20).

C. CHICK EMBRYO WITH CERVICAL FLEXURE FORMED (SIXTY-EIGHT TO SEVENTY-TWO HOURS)

1. *The living embryo.*—Describe carefully the changes visible to the naked eye since the forty-eighth hour. Draw carefully the blood vessels of the vascular area. Remove the chick and the

entire vascular area (keeping the salt solution warm) and with the dissecting microscope note the direction of flow of the blood, indicating same by arrows; arteries carry blood away from the embryo, veins to it. Name the arteries and veins. (See Lillie, Figs. 115, 135.) Make a sketch showing the embryo and blood vessels. Preserve the embryo.

2. *Entire mount.*—A second flexure (*cervical* or *nuchal*) has appeared in the head. The *tail fold* is well formed. What is the position of the embryo on the blastoderm? About the head note three layers: *amnion*, *ectoderm of head*, and *brain wall*. Is the amnion completely closed? Where is the last point of closure? Observe the appearance of an extension of the telencephalon, bilobed anteriorly, rudiment of the cerebral hemispheres; on the ventral surface of the head, a short distance in front of the optic stalks, find the *olfactory pits*. The optic stalks are attached to the floor of the *diencephalon* near the anterior end. The ventral depressed region of the diencephalon behind the optic stalks is the *infundibular region*. The *epiphysis* is a short diverticulum of the roof of the *diencephalon*. The *mid-brain* (*mesencephalon*) forms the apex of the cranial flexure. Where is the cervical flexure located? The mid-brain is united to the metencephalon by the narrow *isthmus*. The most anterior division of the hind-brain with thick roof is the *metencephalon* (rudiment of the *cere-*

bellum and *pons*). The remainder of the hind-brain with thin transparent roof and sides is the *myelencephalon* (rudiment of the *medulla oblongata*). In the *optic cup* observe the inner and outer walls, the *lens*, and the *choroid fissure*. What is the form of the *auditory sac* (*otocyst*)? Above which *visceral arch* does it lie? How many *visceral clefts* are there? The tissue bounding the clefts is thickened to form the *visceral arches*. These are named as follows: (1) *Mandibular*, or *first*, in front of the first cleft (this pair will form the lower jaw); do you find a *maxillary process* arising from the dorsal anterior angle of the arch? (2) *Hyoid*, or *second*, behind the first cleft, (3) and (4) the *third* and *fourth* visceral arches. The first visceral cleft is also known as the *hyomandibular* cleft. The others, if formed, are simply numbered second and third. What is a visceral arch, a visceral cleft, a visceral furrow, a visceral pouch, an aortic arch? Of what adult animal does such a pharyngeal condition remind you? Above the mandibular arch, the rudiment of the *trigeminal* ganglion may be seen; above the hyoid arch, the rudiment of the *acustico-facialis* in contact with the anterior wall of the auditory sac; above the third visceral arch (indistinctly), the rudiment of the ganglion of the *glosso-pharyngeus*. Observe the form and position of the heart and identify *atrium*, *ventricle*, bulbus *arteriosus*, and *aortic arches*. In the trunk observe the

appearance of the anterior and posterior limb buds, the tail bud, etc. How many mesoblastic somites are there? Make a careful drawing of the entire mount.

D. CHICK EMBRYO OF FIVE DAYS

The living embryo.—Great care must be exercised in the removal of the chick from the egg on account of the tendency of the blastoderm to stick to the shell membrane.

How much of the yolk is now covered by blastoderm? Watch very carefully for slight rocking movements of the embryo that are probably due almost entirely to contraction of muscular elements which have developed in the amnion, and serve to prevent adhesions of the embryo with the extra-embryonic membranes. The embryo may be studied while submerged in the *warm* saline solution, and the following observations should be made:

a) The large fluid-filled, saclike structure that may almost or entirely cover the embryo is the *allantois*. Its walls are very vascular and the structure serves as the chief respiratory organ of the growing embryo. How? It arises as a ventral evagination of the hind-gut, growing out through the extra-embryonic body cavity until it completely surrounds the chick. (See Lillie, p. 220, and Figs. 127, 129; also Prentiss and Arey, Fig. 71.)

b) The *yolk-sac circulation* is well developed at this stage. (Compare with Lillie, Fig. 136.)

c) The main blood vessels within the embryo can be seen with the naked eye. Identify: *anterior cardinal* (*jugular*) *vein*, *posterior cardinal*, and the *duct of Cuvier;* also the dorsal aorta. Notice the very abundant blood supply to the brain.

d) The *head*. Identify the parts of the brain. Do you find the *pineal body* (*epiphysis*)? The eye is quite large (characteristic of avian embryos). Is the choroid fissure entirely closed at this stage?

e) By carefully cutting through the transparent membrane (chorion) into the extra-embryonic body cavity (see diagram, Lillie, Fig. 128) the chick surrounded by the amnion is exposed. Carefully turn it over and observe that it is almost entirely free from the blastoderm. What folds in the blastoderm have made this separation possible? The point of attachment remaining is the umbilical cord. Note that all the blood vessels enter the embryo at this point.

Draw the entire vascular area and chick (×3), showing as many of the foregoing features as possible.

E. PIG EMBRYO OF ABOUT 10-MM. NECK-LENGTH

Study in alcohol with the dissecting microscope. (See Prentiss and Arey, Figs. 118, 120.)

a) Lateral view. What is the exact length of your embryo? Measure from the cervical flexure

to the posterior convexity (neck-length). Identify: *amnion*, the transparent membrane surrounding the embryo; *allantois* (previously cut off), a thin, whitish membrane emerging from the umbilical cord; *yolk sac*, a twisted brownish-colored membrane, anterior to the allantois. Carefully strip off the amnion. What is its relation to the body of the embryo and the umbilical cord?

In the head, distinguish *cranial* and *cervical flexures*. Compare these with the 72-hour chick. Determine the position of the myelencephalon, metencephalon, mesencephalon, diencephalon, telencephalon, and the cerebral hemispheres. Compare the *eye* with that of the 3-day chick. Find the *olfactory pits*. Behind the eye observe the large, inverted Y-shaped, *mandibular arch*, the anterior limb of which is the *maxillary process* which fuses with the fronto-nasal process to form the upper jaw; the two mandibular arches fuse ventrally to form the lower jaw. Note position of mouth. The *hyoid arch* is large. The third, fourth and fifth visceral arches are hidden in the *cervical sinus*. Of the visceral furrows only the first or *hyomandibular* (the rudiment of the external auditory meatus) is prominent. The corresponding visceral pouch (first) is the primordium of the middle ear and Eustachian tube. In embryos of 5-mm. neck-length the four visceral clefts are fully exposed. In the stage of 7 mm. the posterior cleft begins to be

covered up by the formation of a pocket-like fold of the integument open in front. In the 8- and 9-mm. stages this closes more and more until only the first visceral cleft is left uncovered. Thus the cervical sinus is formed.

In the trunk observe the *umbilical cord*, the *rudiments* of the limbs, the external molding of the mesoblastic somites, the *milk-ridge* (rudiment of the mammary glands) extending between the bases of fore and hind limbs, and the tail. The post-anal portion of the body (tail) tapers gradually and is not sharply marked off from the trunk. The knob immediately in front of the tail on the ventral surface is the *genital tubercle.* The following internal organs affect the surface contours of the sides and ventral region of the body: the *heart*, the *Wolffian body*, the *liver*, and the embryonic diaphragm. What are the principal external differences at this stage between the avian and mammalian embryo?

b) *The embryo may readily be dissected* under alcohol. Strip off the lateral body wall and remove the limbs. Thus the heart, liver, diaphragm, Wolffian body, and intestine are laid bare. The latter forms a loop, descending deep into the umbilical cord; at the bottom of the loop is the *yolk stalk* that can readily be traced into the *yolk sac.*

Make an enlarged drawing (×10) of your dissection from one side, including the head and visceral arches.

c) *Remove the head* of a 10-mm. pig by an oblique cut behind the cervical sinus. Study with the dissecting microscope.

(1) The mouth is bounded behind by the *mandibular arches*. The antero-lateral boundary on each side is formed by the *maxillary process*, which meets the *fronto-nasal process*, bounding the mouth in front, at a groove (*lachrymal groove*) extending downward and inward from the eye.

(2) The olfactory pits occur at the lateral margins of the front-nasal process. Each is bounded laterally by the *lateral nasal process*, and medially by the *globular* (*median nasal*) *process* of the *fronto-nasal process*. The nasal pits are not clearly separated from the mouth cavity on their inferior aspect.

(3) The cerebral hemispheres project very slightly in front of the fronto-nasal process. (See Fig. 149 Prentiss and Arey.)

Make a drawing of the head from in front.

F. PIG EMBRYO OF ABOUT 15-MM. NECK-LENGTH

Are the flexures as pronounced as before? The *cervical sinus* has closed up and the last trace of the third to the fifth visceral arches thus disappears from the surface. The hyomandibular cleft is more prominent than before. Describe its position and the modification of the bounding arches.

The eye is pigmented. Describe the changes in the limbs since the last stage. What is the condition of the milk-ridge? Note changes in the form of the *genital tubercle* since the stage of 10 mm. Dissect as in the preceding stage. Compare the heart, liver, Wolffian body, and intestine with the 10-mm. stage. Note the very prominent *genital ridge* on the median side of the mesonephros. Draw.

Development of the face: Remove the head and examine it from all sides. Can you still distinguish the fronto-nasal process, the maxillary process, the lachrymal groove, etc.? Is the upper jaw complete? (See Prentiss and Arey, Figs. 124, 149.) What is the embryological foundation for the production of harelip? Draw the head from in front.

G. PIG EMBRYO OF ABOUT 25-MM. NECK-LENGTH

This embryo is distinctly recognizable as a young pig. Compare the cranial and cervical flexures with earlier stages. Describe the *external auditory meatus* (which visceral furrow?), the *external ear* (from which arch?), the eyelid. Observe the beginnings of the large sensory hairs.

Describe the changes in the limbs since the preceding stage. Is the tail definitely developed? What changes in the milk-ridge? *Draw from the side.* Describe the progress in the development of

the snout. Are the elements of the face still distinguishable?

Dissect as before. Compare the relative importance of the Wolffian body with younger embryos. Find the permanent kidney and the genital gland. (See Prentiss and Arey, Figs. 144, 223, 224.) The lungs are readily found. Note the diaphragm and its relations to the heart and liver.

II. EMBRYONIC MEMBRANES, COELOME, MESENTERIES, AND PLACENTA

In this study the sections must be traced rather carefully in order to realize the transformations from one level of the embryo to another. The sections to be drawn may be semi-diagrammatic. The main cavities and structures should be drawn in *outline only*, but must be thoroughly labeled. Different layers of the membranes should be separated sufficiently to be easily followed in the diagram and are to be indicated in colors, as ectoderm, entoderm, and mesoderm. Draw all sections with splanchnopleure ventral and *indicate their levels on the whole mounts by a line across the whole mount drawing.*

A. CHICK EMBRYO OF THIRTY-THREE HOURS

Locate again the section of the chick through the forebrain. The elevation of the somatopleure indicates the lateral folds of the amnion.

In the section through the heart, note that the folds of the amnion have disappeared (this section is posterior to the lateral folds of the amnion). Note the condition of the *coelome*. It is enlarged in the heart region to form the parietal cavity (amnio-cardiac vesicles). Note the dorsal mesocardium. Follow the parietal cavity through the sections to the region of the second or third somites. Is the coelome connected with the parietal cavity? What are the boundaries of the coelome?

B. CHICK EMBRYO OF FORTY-EIGHT HOURS

Locate the following sections and draw in outline only; note especially the condition and extent of amnion, chorion, amniotic cavity, mesenteries of heart and intestine.

a) Select a section (ten to twenty) posterior to the point of the closed amnion, in which the gut is open. The folds of the blastoderm are quite similar to those in the anterior end of the 33-hour chick. The somatopleure is reflected dorsally as a fold, the *lateral amniotic fold*, which is composed of an inner limb (*amnion*) and an outer limb (*chorion*). Identify also the *neural tube*, *notochord*, *dorsal aorta*, *coelome*, *extra-embryonic body cavity*, *somatopleure*, *splanchnopleure*, *gut*, and *lateral limiting sulcus*, the angles where body wall and amnion meet (see Lillie, Figs. 109, 110).

b) Trace the sections from (*a*) anterior or cephalad (note, in passing, the union of the amniotic folds above the embryo—*sero-amniotic connection*) to the region of the sinus venosus. There the somatopleure and sinus venosus have come in contact across the body cavity and have fused to form the *lateral mesocardia* (see Lillie, Figs. 69 and 119). The part of the coelome dorsal to the lateral mesocardium is known as the *pleuroperitoneal grooves*, for into them later grow the lung buds. Through the lateral mesocardia the ducts of Cuvier pass to the sinus venosus of the heart. (Read Lillie, pp. 205–10.) Find the ventral mesentery of the intestine.

c) A section slightly anterior to the lateral mesocardia. This shows the dorsal mesentery of the heart (*mesocardium*) *pericardial cavity*, *pleural grooves*. From this point trace the pleural grooves forward and notice that they shortly end. How related to the pericardial cavity?

d) Through the tenth to twentieth section of your embryo.[1] Is the amnion connected with the chorion? What is the extent of the extra-embryonic body cavity? Are there any mesenteries in this section?

e) Trace through the extreme posterior end of *the embryo*. Has the tail fold been formed in your embryo? Identify the short hind-gut if present.

[1] Not counting sections of the blastoderm anterior to the embryo.

C. CHICK EMBRYO OF SEVENTY-TWO HOURS

It has been seen from the whole mount that the amnion has closed as far back as the region of the posterior limb bud, and that the tail fold of the amnion has grown forward to meet it. In some cases the folds have met and have fused to form an entirely closed cavity, in which later is collected the amniotic fluid. Thus the embryo becomes entirely surrounded by a watery medium in which it develops. Make an outline sketch of the following sections:

a) The fifteenth to twentieth section of *the embryo*. Note that the embryo surrounded by the amnion lies in the extra-embryonic body cavity. The amnion and chorion are separate and each is composed of two layers.

b) The tenth to eleventh section from the posterior end of the embryo. This section should show the posterior end of the embryo free from the blastoderm as in the anterior end of the 33-hour chick. What fold has caused this separation? Identify *amnion*, *chorion*, *somatopleure*, *splanchnopleure*, *body wall*, *extra-embryonic body cavity*. The tail fold of the amnion has formed and extends anterior to this region. Is there a coelome or gut in this section?

c) Trace the sections from (*b*) anteriorly (10 to 20 sec?) to the region where a ventral diverticulum

is given off from the closed hind-gut; this is the beginning of the *allantois* that grows out into the extra-embryonic body cavity and finally surrounds the yolk sac. What is its relation to the ventral mesentery? (See Lillie, Figs. 81 and 127.) Identify and label the principal structures. Follow the allantois anteriorly and see that it ends blindly; how many sections from (*c*)?

d) Trace the sections from (*c*) anteriorly, to the region of the open gut. As the sections are followed from (*c*) to (*e*) identify the posterior intestinal portal (the point of entrance from the yolk sac into the closed hind-gut) and the sero-amniotic connection. The blood vessels lateral to the dorsal aorta are the *postcardinal veins.* Draw a section halfway between (*c*) and (*e*).

e) Through the anterior intestinal portal (the point of entrance from the yolk sac into the fore-gut). Note especially the coelome and mesenteries. The blood vessels in the splanchnopleure lateral to the intestine are the *omphalomesenteric veins.*

f) Trace the sections forward to the *lateral mesocardia* and the openings of the *ducts of Cuvier* into the *sinus venosus* of the heart. The *pleural cavities* (grooves) lie decidedly dorsal to the *pericardial cavity* but are continuous with it anterior to the lateral mesocardia, and both of these cavities are continuous with the *peritoneal cavity* (*coelome*)

behind the lateral mesocardia. Verify this by following the sections slightly forward and backward. Note *dorsal* and *ventral mesenteries* of the intestine. Outline an entire section through the lateral mesocardia, including the amnion and chorion. Over how many sections anterior to (*f*) do the pleural and pericardial cavities extend?

D. PIG EMBRYO OF 10-MM. LENGTH

Since the embryonic membranes have been removed, only the body cavities, mesenteries, and the embryonic part of the allantois will be considered at this time. Make outline drawings and identify the principal structures appearing in the following sections:

a) A section through the posterior part of the anterior limb bud. The section should pass through the stomach, mesonephros, and tip of the heart. (See Prentiss and Arey, Fig. 138.)

The tip of the heart (ventricle) lies free within the pericardial cavity, which at this stage is entirely separate from the *peritoneal* and *pleural cavities*. Verify this by tracing the sections anteriorly. The condensed layer of mesenchyme between the liver tissue and heart cavity is the *embryonic diaphragm*. Lateral to the dorsal aorta is the *mesonephros*. Immediately ventral to the dorsal aorta is the dorsal mesentery of the stomach (the *mesogaster*). Note the *plica venae cavae*, the attachment

of the right dorsal lobe of the liver to the mesogaster, in which is located the *inferior vena cava.* The small cavity between the plica venae cavae and mesogaster is the lesser peritoneal sac. Observe that the *liver tissue* has caused a great expansion of the *ventral mesentery* of the intestine; identify *lesser omentum.* Note the extent of the *coelome.*

b) Trace the series anteriorly (cephalad) until two small cavities appear lateral to the intestine (oesophagus) and lined by the same type of epithelium. These are the lungs (see Prentiss and Arey, Figs. 136, 137), the primordium of which was derived from the distal end of a ventral furrow of the fore-gut. The median part of the coelome into which the lungs project is the *pleural cavity.* This cavity is now separated from the pericardial cavity by the *septum transversum* (embryonic diaphragm) derived in part from the lateral mesocardia. See that it is still connected with the peritoneal cavity (if not in this section, in one anterior or posterior). Follow the pleural cavities forward and note that they end blindly.

c) Through the umbilicus. Select this section to pass through the point at which both coelome and dorsal mesentery supporting the intestine project out into the umbilicus. The *peritoneal cavity* alone is represented in this section. The two veins in the body wall just ventral to the lower

angle of the mesonephros are the *umbilical veins;* notice that the left one (apparent right) is much the larger. The right one shortly disappears. Ventral to these are the two *umbilical arteries*, almost the same size, and between these the small angular cavity of the *allantoic stalk.* At the ventral median angle of the mesonephros note the *mesonephric duct.*

d) Trace the allantois posteriorly until it opens into the *urogenital sinus.* Sketch the entire section at this point. (See Prentiss and Arey, Fig. 122 and 141.)

E. DISSECTION OF PIG PLACENTA[1]

The pig uterus is of the bicornuate type, consisting of two *uterine horns* meeting posteriorly to form the *body* of the *uterus*, the cavity of which continues through the *cervix uteri* into that of the *vagina.* Distinguish the *vagina*, *cervix*, body of uterus (*corpus uteri*), horns of uterus (*cornua uteri*), *Fallopian tubes*, and *ostium tubae abdominale.* Note the position and attachment of the *broad ligament* by which the uterus is suspended in the body cavity. At the anterior end, each horn of the uterus continues as the small, thick-walled Fallopian tube ending in a thin-walled sac, surrounding the *ovary* except for a terminal aperture into the body cavity, the *ostium tubae abdominale.* Escaping ova are taken up by the widened, membranous end of the

[1] A pregnant sow's uterus should be furnished each two students for the following exercise.

tube and transported to the horns of the uterus, where development is carried on.

The clear vesicles on the surface of the ovary are *Graaffian follicles* containing ovocytes. The larger yellow protrusions are *corpora lutea* (so called from their slightly yellowish color); one is formed from each Graaffian follicle after ovulation and they are thought to function as organs of internal secretion. Cut open a corpus luteum and note its solid character. How do the number of embryos in the uterus correspond with the number of corpora lutea in both ovaries?[1]

The placenta of mammals in general is a compound structure formed by apposition or fusion of a *maternal part* (uterine mucosa) and a *foetal part* (the chorion and allantois).

Very carefully cut a small slit in the wall of the uterus without injuring the embryonic part of the placenta, which is held against the walls of the uterus by the contained fluid. The uterine wall can now be torn by slight pulling, with less liability of tearing the chorion than by cutting. Identify the *muscular layer* and *mucosa* of the uterus and note the position and attachment of the blood vessels. Expose the entire extent of one of the large fluid-filled vesicles containing the embryo; it is a chorionic vesicle, the outside layer of which

[1] The corpora lutea of pregnancy are much larger than are those found in the absence of pregnancy.

is the *chorion*. Remove one entire vesicle and float in water. How are these vesicles held in place within the uterus? Such a condition, in which there is no localized development of special areas of union between the chorion and the uterine mucosa, is termed *diffuse placentation*.

The pig placenta is a type known as *Placenta epithelio-chorialis* from the fact that the epithelium of the mucosa of the uterus persists throughout pregnancy in contact with the chorion, or embryonic part. It will be realized that substances carried in the maternal blood in order to reach the blood of the foetus must pass through the following membranes: (1) endothelial lining of maternal blood vessels, (2) connective tissue of uterus, (3) uterine epithelium, (4) space, (5) chorionic epithelium, (6) connective tissue of chorion, (7) endothelial lining of foetal blood vessels.

With scissors cut through the walls of the chorionic vesicle (chorion and allantois), from one end to the other, into the fluid-filled cavity of the *allantois* which has expanded sufficiently to eliminate the cavity of the chorion. Reflect the cut edges of the membranes and note that the amnion is covered by the outer wall of the allantois, the mesodermal layer of which is fused with the mesodermal layer of the amnion. The amnion is visible only through the walls of the allantois. Find the *yolk sac* between the amnion and allantois on the

side of the embryo where the amnion is fused with the chorion. At this point the allantois does not separate amnion from chorion. How is the embryo connected with the foetal part of the placenta (chorion and allantois)?

Prepare diagrams, using colors to distinguish the various layers, of both a longitudinal and a cross-section of the embryo through the umbilical cord to show the relations of the membranes to the embryo.

III. ECTODERMAL DERIVATIVES

Nervous System

The nervous system may be divided into (1) the *central* (brain and spinal cord), (2) *peripheral* (spinal and cranial nerves) and (3) the *sympathetic* systems. The first two, only, will be considered here.

A. CHICK EMBRYO OF THIRTY-THREE HOURS

The principal derivatives of the nervous system can be seen very successfully in the whole mount. Refer to it again and notice the metameric condition of the hind-brain; the segments are termed *neuromeres*. The brain of the chick embryo is regarded as being composed of eleven neuromeres distributed as follows: *forebrain*, first, second, and third; *mid-brain*, fourth and fifth; *hind-brain*, sixth to eleventh.

From the serial sections draw a section through the hind-brain, with a short piece of the accompanying epidermis to show the *neural crest* (Lillie, Figs. 57, 90). What is the neural crest and to what does it give rise? Is the entire spinal cord differentiated at this stage? Give reason for your answer.

B. CHICK EMBRYO OF FORTY-EIGHT HOURS

The five divisions of the brain are now well differentiated. Note the ganglion of the *fifth cranial nerve* (the trigeminus), and the *seventh* and *eighth* (acustico-facialis) which arise in conjunction with each other. The cranial ganglia are products of the neural crest. Notice that the roof of the myelencephalon is decidedly thinner than any other division of the brain, indicating the non-nervous covering of the *fourth ventricle*. Does the neural tube appear to be closed at its posterior end?

a) Draw in outline the entire nervous system of the whole mount embryo and label thoroughly.

b) Study a section through the cord under high power. At this stage the cord is a relatively simple epithelial tube; the central ends of the cells have formed an *internal limiting membrane* while the peripheral ends have given rise to the *external limiting membrane* (See Lillie, Fig. 138). Observe the rounded *germinal cells* inclosed between the

inner ends of the epithelial cells. Do you find mitotic figures in the germinal cells? By multiplication and differentiation these cells give rise to spongioblasts, indifferent cells, and neuroblasts (read Lillie, pp. 233–44). Ependyma, mantle layer, and marginal velum are not yet clearly differentiated, neither have distinctively nervous elements made their appearance.

Draw a section of the cord showing details of the cell-structure.

C. CHICK EMBRYO OF SEVENTY-TWO HOURS

Identify again the principal structures of the nervous system from the whole mount, noting the new structure on the dorsal side of the diencephalon, the *epiphysis*, an organ of uncertain function and significance.

a) Draw a section through the head region showing *myelencephalon*, *metencephalon*, *isthmus*, and *mesencephalon*, *otocysts*, ganglia of *fifth*, and *seventh* and *eighth* cranial nerves (refer to whole mount). To what neuromeres is the trigeminus ganglion attached?

b) Trace the sections to the region of the mesencephalon and note two small nerves growing from its ventral surface into the surrounding mesenchyme; these are the *third* or oculo-motor cranial nerves.

c) The *hypophysis*, a compound structure partly nervous (*posterior lobe* from the infundibulum, a

pocket in the floor of the diencephalon) and partly epithelial (*anterior lobe* from oral cavity), is present in its components at this stage. (See Lillie, Figs. 85, 87, 88, and 101.) Identify these structures and *draw* a section showing anterior and posterior lobes.

D. PIG EMBRYO OF 10-MM. LENGTH

Select sections through the following regions and draw:

a) Thirtieth to fortieth section of the series to show the thin dorsal wall of the myelencephalon; the cavity of the myelencephalon is the *fourth ventricle.*

b) A section through the ventral tip of the otocyst (auditory vesicle) showing mesencephalon, metencephalon, myelencephalon, fifth, seventh, eighth, ninth, tenth cranial nerves. (Read Lillie, pp. 261–70, and see Prentiss and Arey, Fig. 120.)

Identify also the third or oculo-motor nerve from the ventral surface of the mesencephalon. Can you identify the eleventh and twelfth cranial nerves? Follow the fifth nerve and see that it divides into three branches: (1) the opthalmic branch running toward the eye, (2) the maxillary branch running to the maxillary process, and (3) the mandibular branch running into the mandibular arch. Find also the anterior and posterior lobes of the hypophysis.

c) A section through the tip of the head showing the two lobes from the telencephalon, the *cerebrum;*

the cavities of these are the lateral, or first and second ventricles.

d) A detailed section through any part of the spinal cord showing the relations of a spinal nerve to it. Note the *dorsal* and *ventral zones* of His (sensory and motor areas). The wall of the neural tube is composed of three layers, (1) the *ependymal layer* bordering the central canal, including the large *germinal cells*, (2) the *mantle layer*, the densely nucleated part of the walls of the tube, composed of cells that become spongioblasts and neuroblasts—the gray matter of the cord—and (3) the *marginal velum*, the external non-nucleated layer composed of nerve processes—the so-called white matter. Note especially the *motor neuroblasts* ventro-laterally. Each spinal nerve is composed of two roots: (1) a *dorsal* or *sensory root*, the fibers of which arise from the ganglionic neuroblasts located in the *dorsal ganglion* outside of the neural tube, central processes growing into the cord, and (2) a *ventral* or *motor root*, the fibers of which arise from the medullary neuroblasts located within the ventral or motor horn of the cord. Peripherally the spinal nerves divide into three main branches: (1) the dorsal branch, passing dorsally just external to the ganglion; (2) the ventral branch passing into the somatopleure; and (3) the visceral branch (ramus communicans) passing toward the aorta from the dorsal angle of the body cavity. (See Prentiss

and Arey, Fig. 360.) From what do the ganglionic and medullary neuroblasts arise? (See textbooks.)

Organs of Special Sense

Olfactory:

The primordium of the olfactory apparatus is found at first as a thickened plate of ectodermal cells (olfactory placode) on the ventro-lateral side of the head. This plate of cells later invaginates in such a manner that the grooves produced by the invagination connect with the oral cavity. By the fusion of the maxillary process with the ventro-lateral ends of the median frontal process the external nares are produced.

A portion of the invaginated placode or nasal epithelium is transformed into sensory olfactory epithelium, certain cells of which (epithelial neuroblasts) send processes centrally to connect with the brain. Make an outline sketch of a section of the head of the 72-hour chick showing the nasal fossae (olfactory pits) and the thickened epithelium. Study this also in the 10-mm. pig. Describe.

Auditory:

The ear is a compound structure composed of three parts: (1) inner ear—membranous labyrinth (utriculus, sacculus, cochlea, semicircular canals, etc.) developed from ectoderm of the head (otocyst) and surrounded by the osseous labyrinth produced

by ossification of the adjacent mesenchyme; (2) middle ear—tympanic cavity and Eustachian tube (tubo-tympanic canal) from the first pharyngeal pouch (entoderm) and auditory ossicles from surrounding mesoderm; (3) external ear—auricle and external auditory meatus from first and second visceral arches and first visceral furrow (ectoderm). The tympanic membrane (tympanum) is produced by a thinning of the tissue between (2) and (3) and is composed of all three germ layers.

Membranous labyrinth:

A. CHICK EMBRYO OF FORTY-EIGHT HOURS

Sketch a section through the open otocyst (auditory vesicle). It is formed as an invagination of ectoderm of the head, but is surrounded by mesoderm which later forms a bony capsule (the osseous labyrinth) around the otocyst complex.

B. PIG EMBRYO OF 10-MM. LENGTH

Near the point of closure of the otocyst a median dorsally directed outgrowth is formed, the *endolymphatic duct* (recessus labyrynthi), that projects above the dorsal surface of the otocyst. *Draw* part of a section showing the opening of the endolymphatic duct into the otocyst. Trace the sections through the otocyst and note the innervation of it by the eighth cranial ganglion. The separation into utriculus and sacculus from which come the

semicircular canals and cochlea is not yet accomplished, though indicated by dorsal and ventral parts. (Consult textbooks.)

Middle ear, external ear, and tympanic membrane:

A. PIG EMBRYO OF 10-MM. LENGTH

Identify the first pharyngeal pouch which may be found near the lower boundary of the ganglion of the seventh cranial nerve. Trace the cavity of this pouch (posteriorly in the series) to the point at which the pouch opens into the pharynx.

The first pharyngeal pouch is the primordium of the *tympanic cavity*, and the narrow tube connecting the pouch with the pharynx is the primordial *Eustachian tube*. The ectodermal groove represents the future *external auditory meatus*. Between the ectodermal groove and the outer (entodermal) wall of the tympanic cavity is a layer of mesenchyme; these three layers compose the primordium of the *tympanic membrane* (tympanum). *Draw* and thoroughly label a complete section to show these structures.

Optic:

The eye is a structure developed partly from the nervous system (retina), partly from ectoderm of the head (lens), and partly from mesoderm (choroid coat, sclerotic coat, etc.).

A. CHICK EMBRYO OF THIRTY-THREE HOURS

From a section through the primary optic vesicle (continuous with the cavity of the brain) observe that the peripheral wall of the vesicle is in contact with the ectoderm of the head. Is this ectoderm thickened? The cavity of the optic vesicle (ventriculus opticus) is continuous with the ventricle of the fore-brain.

B. CHICK EMBRYO OF FORTY-EIGHT HOURS

Observe from sections that the primary optic vesicle has invaginated, resulting in a partial obliteration (later complete) of the cavity and the production of a secondary cavity (cavity of *vitreous humor*) opening externally; it is now called the *optic cup*. The two layers of the optic cup formed by this invagination give rise to the retina proper (inner layer) and the pigment layer of the retina (outer layer). Note that the invagination of the optic vesicle involves the floor as well as the external wall of the vesicle, thus producing the choroid fissure as well as the pupil. The connection of the optic vesicle with the brain, the *optic stalk*, is now very much constricted. The *optic placode* (thickened ectoderm of the head) has also invaginated to form the *lens vesicle*, which lies within the opening of the optic cup (pupil of the eye). The space between the lens and the retina will be the

cavity of the *vitreous humor*. *Draw* a section of the head to show the open lens vesicle.

C. CHICK EMBRYO OF SEVENTY-TWO HOURS

a) Identify all the above-mentioned structures found in the 48-hour chick. The ventral wall of the optic cup is invaginated, from pupil to stalk, and a temporary fissure is produced through which mesenchyme and blood vessels may enter; this temporary fissure is the *choroid fissure* (see Lillie, Fig. 96). If this fissure fails to close, the condition of *coloboma iridis* is produced. *Draw* a section through the choroid fissure. Identify it on the whole mount.

b) The walls of the lens vesicle, at first of equal thickness, have now begun to undergo differentiation into a thick inner layer that gives rise to lens fibers and a thin outer layer, the epithelial layer of the lens. Draw a lens vesicle under a high magnification. Is the epithelium stratified?

D. PIG EMBRYO OF 10-MM. LENGTH

Draw a section of the head showing the *choroid fissure* of the eye. Can you distinguish the central artery of the retina? Observe the layer of mesenchyme between the ectoderm of the head and the lens. This is the site of the future *anterior chamber* of the eye. The *cornea* is produced from ectoderm of the head and mesoderm. The *sclerotic*, *choroid*

coat, and other accessories arise principally from mesoderm surrounding the optic vesicle. The *pupil* is represented by the open outer part of the optic cup occupied by the lens.

IV. ENTODERMAL DERIVATIVES

Digestive Tract and Appendages

The formation of the head fold produces an internal bay in the entodermal layer of the blastoderm which is the primordium of the fore-gut; that of the hind-gut is similarly formed by the tail fold of the embryo, while the walls of the mid-gut diverge and are continuous with the yolk sac. Embryologically the fore-gut is considered as that part of the alimentary tract from the liver diverticula anteriorly, and includes the pharynx, esophagus, stomach, duodenum, and the following outgrowths: visceral pouches, tongue, thyroid, parathyroid, and thymus glands, respiratory tract, liver, and pancreas. The mid-gut is that portion between the liver and coecal appendages. The hind-gut is the part of the tract from the coecal appendages posteriorly and gives rise to the coecal appendages, colon, rectum, cloaca, allantois, and anal plate (in part).

It will be understood that the entodermal tissue gives rise merely to the lining epithelium of these parts and that mesenchyme accompanies all the

structures produced. The student should refer constantly to the whole mount for an interpretation of the section under consideration.

A. CHICK EMBRYO OF THIRTY-THREE HOURS

Refer again to the section through the anterior intestinal portal. Posterior to this region in any section, the lower layer of entoderm represents the dorsal wall of the intestinal tract (see Lillie, Fig. 67). What is the anterior intestinal portal?

B. CHICK EMBRYO OF FORTY-EIGHT HOURS

a) *Draw* a section through the upper part of the pharynx showing the *first visceral pouch* and the *oral plate*. The oral plate is a strip of tissue (entoderm of pharynx and ectoderm of oral cavity) between the two mandibular arches that separates the pharynx from the oral cavity. Is the *anterior lobe* of the *hypophysis* shown in this section? The blood vessels in the mandibular arches are the first aortic arches. Note the *visceral groove;* the *closing membrane*. Is the first visceral pouch open to the outside (visceral cleft)?

b) Trace the intestine posteriorly and identify all the pharyngeal pouches. How many are present in your series? Follow the sections to a point just in front of the anterior intestinal portal, to the ventral diverticulum growing out into the ventral mesentery. This is the *anterior liver divertic-*

ulum. Does it grow anteriorly or posteriorly from its point of origin? Trace the cavity to see that it ends blindly.

Draw a section through its point of origin. The blood vessel immediately below it is the *ductus* (*meatus*) *venosus.* The posterior liver diverticulum is just beginning at this stage.

c) Over how many sections does the hind-gut extend in your series?

C. CHICK EMBRYO OF SEVENTY-TWO HOURS

a) Identify in the pharyngeal region, the *oral cavity*, *oral plate*, *anterior lobe* of the *hypophysis*, and all the *pharyngeal* (*visceral*) *pouches* (how many?). The *thyroid gland* arises as a ventral diverticulum from the floor of the pharynx between the bases of the second and third pair of visceral arches. Are the visceral pouches open at this stage? Which ones?

Draw a section showing the thyroid and as many visceral pouches as possible. Label thoroughly.

b) Posterior to the visceral pouches there is a deep ventral depression of the pharynx, the *laryngo-tracheal groove*, which gives off two ventrolateral diverticula, the *lung buds.* (See Lillie, Fig. 97.)

c) Trace the sections through the remaining part of the fore-gut, identifying the *anterior* and *posterior liver diverticula;* the latter lies ventral to the ductus venosus and connects with the intestine

posterior to the former. Note the ramifications of the liver diverticula around the ductus venosus. The *dorsal pancreas* is an outpouching of the intestine opposite the origin of the posterior liver diverticulum; it will be seen as a small thickened mass of cells with or without a small lumen.

Draw a section showing the pancreatic diverticulum.

d) Identify the hind-gut, which is closed at this stage, and note again the allantoic diverticulum which grows out into the ventral mesentery. Identify the *cloacal membrane*, where entodem of hind-gut is fused with ectodem. Find the post anal gut (see Lillie, Fig. 80).

e) With the aid of the whole mount and Lillie's Fig. 100 prepare a diagram of a lateral view of the digestive system showing fore-, mid-, and hind-gut. Place guide lines at the side of the diagram with the number of the section of your series in which the following structures appear: the dorsal wall of the pharynx, anterior lobe of the hypophysis, origin of the anterior liver diverticulum, pancreas, posterior intestinal portal, and origin of allantois.

D. PIG EMBRYO OF 10-MM. LENGTH

a) Identify again the first pharyngeal pouch (primordium of tympanic cavity). The second and third visceral arches are then easily recognized. The blood vessel in the third visceral arch

is the *third aortic arch.* Continue posteriorly (in the series) to a point below the level of the pharynx between the bases of the second and third visceral arches and note the thyroid gland (a small dark mass of tissue) in the general mesoderm; by this time it has severed its connection with the pharynx. What is the thyreo-glossal duct? Identify the third pharyngeal pouch and the fourth visceral furrow (the fourth pouch appears more posteriorly). On the anterior face of the third visceral pouch will be seen a thickened mass of cells; this is one primordium of the *epithelial bodies* (*parathyroid glands*). Proceeding posteriorly (a few sections) see that the cavity of the third visceral pouch extends ventrally as a blind pocket directed medially; this ventral diverticulum of the third pouch is the primordium of the *thymus gland.*[1] The *laryngotracheal groove* will probably appear in this region as a ventral groove of the pharynx. *Draw* a section through the thyroid gland showing, if possible, the thymus, epithelial bodies, anterior lobe of the hypophysis, and the laryngotracheal groove.

b) *Draw* a part of each section to show the following structures and their relations:

1. *Fourth visceral pouch* and *laryngotracheal groove.* What is the glottis?

[1] The paired thymus later unites to form an unpaired gland located ultimately in the upper part of the thorax.

2. *Esophagus* and *trachea* through the bifurcation of the bronchi.

3. Through the *stomach*, to show entodermal lining (epithelium of future mucosa), mesenchymal layer (future connective tissue of mucosa, submucosa, and muscle layers), and mesodermal covering (serous layer).

4. The dorsal pancreas.[1] Identify also the ventral pancreas. Trace the sections through the liver noting again the original hepatic diverticulum, an epithelial tube opening into the gut; this is the primordium of the common bile duct and gall bladder (see Prentiss and Arey, Figs. 139 and 185).

5. Trace sections through the mid-gut, hind-gut, and cloaca. Note the relations of the yolk stalk, allantois, and cloacal membrane. What is Meckel's diverticulum? What is the urachus? *Draw* a section through the cloacal membrane.

[1] At this stage both the dorsal pancreas (duct opening into duodenum) and ventral pancreas (duct opening into common bile duct) are present, and separated by the portal vein. Later these two parts fuse, the ventral pancreas forming a part of the head and uncinate process while the dorsal pancreas forms the remainder of these parts as well as the entire body and tail of the adult pancreas.

In the pig the duct of the dorsal pancreas may persist as the functional duct, while in man the ventral duct persists, being the duct of Wirsung; in man the duct of the dorsal lobe usually remains patent, being the accessory pancreatic duct or duct of Santorini.

V. MESODERMAL DERIVATIVES

Urogenital System

The urinary system and the genital organs and ducts are closely associated in development and will be considered together.

In the amniote vertebrates three sets of kidneys are formed during development: (1) the pronephros, probably homologous to the permanent kidney of *Amphioxus*, (2) the mesenephros, the functional kidney of anamnia (frog, etc.), and (3) the metanephros, or adult kidney. All three kidneys are derived from the differentiation of the nephrotome or intermediate cell mass.

1. *The pronephros* (*head kidney*):

The pronephros in both birds and mammals is probably never functional, and is characterized by its position (fifth to fifteenth somite in chick, seventh to fourteenth in human) and by the fact only one tubule arises in each segment. The medial end of a pronephric tubule connects with the coelome by an opening (nephrostome) and the distal end opens into the pronephric duct; a glomerulus is present, but owing to the absence of a Bowman's capsule a Malpighian corpuscle does not exist.

A. CHICK EMBRYO OF THIRTY-THREE HOURS

Study (under high magnification) a section three or four somites in front of the last one formed.

Notice the mass of cells on the dorsal surface of the *intermediate cell mass* (*nephrotome*) between the *lateral plate* and the *somite.* Does it possess a cavity or is it solid? This is the pronephric duct.

A dorsal projection grows out from the nephrotome in each segment (5–15) and continues (blindly) posteriorly to the next segment, where it unites with the tubule of that one and so gives rise to a continuous duct, the *pronephric duct*, which continues its growth posteriorly, reaching the cloaca about the stage of thirty-one somites. The pronephric duct becomes also the duct of the mesonephros (Wolffian duct).

2. and 3. *The mesonephros and metanephros:*

The mesonephros is composed of a mass of tubules also produced from the intermediate cell mass, and is characterized by its position (fourteenth to thirtieth somite in chick, thirteenth to twenty-eighth in human), by the formation of Malpighian corpuscles, by the absence of nephrostomes, and by the fact that several tubules arise in the same segment.

A. CHICK EMBRYO OF FORTY-EIGHT HOURS

Select a section about the region of the twentieth somite and note the thick-walled mesonephric duct (Wolffian duct) and a more median mass of darkly stained cells, the *nephrogenous tissue;* the latter will be converted into the tubules of the meso-

nephros. First, balls of cells arise within the nephrogenous tissue, each ball later forming a vesicle, and finally the vesicles elongate into tubules and open into the Wolffian duct laterally. Find these openings; the blind median end will later form a Malpighian corpuscle. In the 48-hour stage these processes are just beginning in the anterior part of the mesonephros.

B. CHICK EMBRYO OF SEVENTY-TWO HOURS

The mesonephric tubules are very distinct at this stage; note that more than one opens into the Wolffian duct in a segment. A considerable amount of the nephrogenous tissue, situated dorsally to these tubules, is not yet converted into tubules; secondary and tertiary tubules arise from it later. *Draw* a section (under high power) showing the opening of one of these tubules and the relation to the dorsal aorta, postcardinal veins, and coelome.

C. PIG EMBRYO OF 10-MM. LENGTH

a) The mesonephros is fully formed. The mass of mesonephric tubules is known as the Wolffian body or mesonephros (which is probably functional in the chick and pig during embryonic development). Study a section toward the posterior end of the mesonephros, noting the very much coiled *mesonephric tubules* and the *Wolffian duct* at the ventral border into which the tubules empty.

Each tubule begins in a renal (or Malpighian) corpuscle situated medially, consisting of a thin-walled capsule (Bowman's capsule) continuous with the secreting tubule, and a glomerulus or vascular invagination of the capsule. The coiled secretory tubule connects with the Wolffian duct by a short conducting portion. Find the postcardinal vein at the dorsal side of the mesonephros; note, also, branches of the dorsal aorta entering the corpuscles. Find the thickened ridge of the peritoneum on the median side of the mesonephros between the Wolffian duct and the dorsal mesentery. This is the *genital fold* that later becomes transformed into the gonad. *Draw* a section of the Wolffian body.

b) Trace the Wolffian duct until it opens into the cloaca. A diverticulum of the duct will be found that extends dorsally and anteriorly from its origin near the point at which the Wolffian duct enters the cloaca; it is the *metanephric duct* (ureter). Trace the duct and see that the cavity expands considerably and ends blindly. The expanded portion is the primitive pelvis of the metanephros; the condensed, darkly stained nephrogenous tissue (*metanephric blastema*) surrounding the primitive renal pelvis is quite distinct. At a later stage the metanephric duct branches into several sets of tubules, the whole structure becoming the medullary part of the adult kidney (the pelvis, calyces, and collecting tubules); the secreting tubules of the kidney owe their origin

to the nephrogenous tissue covering the branching tubules of the original diverticulum (consult Lillie, McMurrich, and Prentiss and Arey). The metanephric duct becomes the ureter of the adult. *Draw* a section showing the primitive renal pelvis and metanephric blastema; *make also* an outline diagram (lateral view) showing the relations of the primitive metanephros and duct to the mesonephros, mesonephric duct, and cloaca.

The Genital System

The gonads are produced from the germinal epithelium of the genital folds. The Wolffian duct becomes the *vas deferens* of the male and disappears in the female. A new tube, the Müllerian duct, arises later from the lateral side of the mesonephros (the anterior end retaining an open connection with the coelome), which in the female becomes the oviduct, uterus and vagina, but in the male disappears except for certain functionless rudiments. Consult textbooks on these points, on the urogenital sinus, and formation of the ducts in the female.

Circulatory System

The entire circulatory system, from our present knowledge, is to be classed as a derivative of mesoderm.

The first elements of the system to be seen in the chick are the blood islands in the germ-wall; the peripheral cells of these islands are transformed

into the endothelial lining of the blood vessels, while the central cells become erythrocytes. Early in the formation of the vascular system cavities arise apparently in situ from mesoderm, throughout the splanchnopleure and embryo; by extension and anastomosis the cavities become connected, and establish a vascular network, first on the yolk sac in connection with the blood-islands and later within the embryo.

The heart:

The heart is formed by the approximation and fusion of two lateral endothelial tubes (endocardium) which are surrounded by thickened layers of the splanchnic mesoderm (myocardium) that gives rise to the muscular part of the heart.

A. CHICK EMBRYO OF THIRTY-THREE HOURS

The whole mount shows the heart as a comparatively straight tube into which the omphalo-mesenteric veins enter. The anterior end continues as a straight tube that divides into the two ventral aortae. Review these relations again from the whole mount and sections.

B. CHICK EMBRYO OF FORTY-EIGHT HOURS

Study the heart from the whole mount. Since the anterior and posterior ends of the heart are attached and the tube increases greatly in length, a fold to the right is the result, causing the heart to slightly resemble the letter S. Constrictions

in the tube have begun to form and give indications of the various parts—bulbus arteriosus, ventricle, atrium, and sinus venosus. From the sections identify the above-named chambers.

Draw a section showing the cavity of the ventricle continuous with that of the atrium, noting the wide separation of the endocardium from the myocardium in the former. This separation is characteristic for the bulbus and ventricle and the close application of the two for the sinus and atrium. The space between these two layers will later be filled in by a thickening of the myocardium, giving rise to the muscular part of the heart.

C. CHICK EMBRYO OF SEVENTY-TWO HOURS

Note the position and relation of the various parts of the heart on the whole mount. Definite constrictions are present that separate the structure into the four chambers, and by a concrescence of the omphalomesenteric veins a single venous channel, the *ductus venosus*, enters the heart from behind. Note that the bulbus arteriosus lies on the right side (apparent left). Identify the heart in all its parts and *draw* a section showing the communication of the sinus venosus with the atrium.

D. PIG EMBRYO OF 10-MM. LENGTH

At this time the two auricles, derived from the primitive atrium, and the two ventricles, derived

from the early single ventricle, are in process of formation.

The sinus venosus is almost entirely incorporated in the right auricle. Find the two valves that guard the opening of the sinus into the auricle (see Prentiss and Arey, Figs. 134, 260). The *endocardial cushion* is a mass of tissue (much lighter in color than the other parts) in the auriculo-ventricular canal, which it divides into right and left passages. See that the *auricular septum* has fused with it ventrally; find the *foramen ovale* (the secondary perforation of the auricular septum). Note also the *interventricular septum*, and see that it does not yet completely separate the two ventricles. *Draw* a favorable section showing the two auricles and ventricles.

A septum has formed within the bulbus, beginning at the upper end and growing toward the ventricles, so as to divide it into two longitudinal vessels that emerge from the ventricles together (the septum is not yet complete along the entire extent of the bulbus); one of these vessels is the systemic aorta, the other the trunk of the pulmonary arteries. *Draw* a cross-section showing these two trunks.

The Arterial System:

A. CHICK EMBRYO OF THIRTY-THREE HOURS

The endothelial lining of the heart is continued anteriorly as a single vessel, the *ventral aorta*, that

bifurcates at the level of the oral plate, giving rise to the first pair of aortic arches that arch around the pharynx, through the mandibular arches, and continue posteriorly as the dorsal aortae (descending aorta). Follow the dorsal aorta posteriorly; each ends in a network of vessels that are continuous with the network in the area vasculosa.

B. CHICK EMBRYO OF FORTY-EIGHT HOURS

In the 48-hour chick additional connections of the ventral aorta with the dorsal aorta have been formed in the second pair of arches. From the bulbus, trace the sections anteriorly, noting the origin of the aortic arches from the ventral aorta, their course through the corresponding visceral arches, and their union with the dorsal aorta on each side. What aortic arches are present in the 48-hour chick?

By approximation and fusion, the two dorsal aortae found in the 33-hour chick have been converted into a single median aorta posterior to the heart. Note the intersegmental branches. The aorta continues posteriorly to about the center of the mid-gut, where it is again double, and each vessel gives off a lateral branch, the *vitelline* (*omphalomesenteric*) *artery*. *Draw* a section through the origin of the vitelline artery. The two dorsal aortae then continue posteriorly, giving off numerous segmental arteries, and gradually become smaller until they disappear near the

posterior end of the embryo. Trace them through their entire extent.

C. CHICK EMBRYO OF SEVENTY-TWO HOURS

From the bulbus arteriosus, follow the single ventral aorta (anteriorly in the series) to the point at which the aortic arches course around the pharynx, running through their respective visceral arches. How many aortic arches in your series? Which ones are they? Trace each aortic arch to its union with the dorsal aorta on each side. The anterior part of the dorsal aorta supplies the head more anteriorly and constitutes part of the future *internal carotid artery*. The union of the two dorsal aortae occurs just posterior to the opening of the last aortic arch (fourth) into the aorta. Find the roots of the vitelline arteries. Do they come off from a single or double aorta? Trace the two aortae from this point to their termination.

Draw a lateral view of the arterial system, using the whole mount (for outline) and sections. Place guide lines at the side and indicate the position of the following parts by the number of the section in your series in which they occur: (1) Base of third aortic arch, (2) union of second arch and dorsal aorta, (3) internal carotid artery at level of posterior lobe of hypophysis, (4) point of union of two dorsal aortae, (5) through vitelline arteries, (6) posterior termination of aortae.

D. PIG EMBRYO OF 10-MM. LENGTH

The embryonic condition of the aortic arches is being gradually changed over into the adult condition. In the 10-mm. pig the first and second arches have lost their connections with the dorsal aorta and appear only as very small vessels arising from the base of the third pair of arches. They go to make, later, the external carotid artery (see Prentiss and Arey, Fig. 273). The third aortic arch retains its connection with the dorsal aorta. The latter, however, is decidedly smaller between the openings of the third and fourth arches and will disappear later, leaving that part of the dorsal aorta anterior to the third arch as the internal carotid artery, the base of the arch itself being the common carotid. The fourth arch also retains its connection with the aorta and will (later) become, on the left, the arch of the systemic aorta; on the right it will persist only part way to become the subclavian artery of the adult. The fifth arch is transitory and will not be seen, while the sixth is connected with the aorta on both sides and gives off a branch running posteriorly from the arch, the pulmonary artery.

See directions (at end of this section) for the outline to be made of the arterial system and record the number of the sections in which the named points occur at the time of identification.

The bulbus arteriosus, previously drawn, has been divided distally by a septum into (1) the pulmonary trunk on the left (apparent right), and (2) the arch of the systemic aorta on the right. Follow these trunks anteriorly to where the former gives off a pair of vessels that arch around the pharnyx to connect with the dorsal aorta on each side. From the diagram and reference to the whole mount, it will be realized that these are the posterior arches or the sixth pair. Return posteriorly (in the series) to the lung diverticula, and by tracing the sections anteriorly two small vessels will be found lying immediately below the trachea that can be traced to their origin from the sixth pair of arches. These are the *right* and *left pulmonary arteries*. The part of the sixth arch between the origin of these and the dorsal aorta is the *ductus arteriosus* (*duct of Botallus*), which disappears when the lungs begin to function.[1]

Return again to the base of the sixth arches and a cross-section of the systemic aorta will be seen. Follow this anteriorly, until two pairs of arches are given off very close together, running dorsally to open into the aorta; these are the *third* and *fourth arches*. From the base of the third arch

[1] The ductus arteriosus on the right side disappears comparatively early in development but persists on the left side until birth. It then ceases to function, but remains in the adult as the ligamentum arteriosus connecting the pulmonary artery with the aorta.

and just at the angle of the thyroid find the small branches that run toward the *second* and *first* visceral arches; these are the remains of the *second and first aortic arches* that become the external carotids. Trace the fourth and third arches through their corresponding visceral arches, into the dorsal aorta and note the small size of the latter between the two. Blood in the fourth arch is directed posteriorly, while that in the third is directed anteriorly; consequently the aorta between these very soon disappears. From the third arch follow the aorta (internal carotid) into the head; although rather small it can be easily traced. The *internal carotid* will be found to run forward to the walls of the diencephalon just lateral to the anterior lobe of the hypophysis, to turn dorsally and posteriorly, meeting its partner from the opposite side to form the *basilar artery* immediately underneath the mesencephalon (see Prentiss and Arey, Fig. 276 and p. 264). The *basilar artery* continues posteriorly beneath the myelencephalon and divides into the two *vertebral arteries*, each of which runs posteriorly at the ventro-lateral angle of the neural tube.[1]

[1] In some embryos it may be impossible to find definite vertebral arteries as they are in the process of formation at this stage. This anastomosis of the internal carotids and vertebral arteries establishes the double arterial blood supply for the brain; from this the circle of Willis (on ventral surface of brain) of the adult is established (consult Cunningham's anatomy).

The dorsal aorta: Near the anterior end of the Wolffian bodies the two dorsal aortae meet to form the single aorta. This continues posteriorly and gives off dorsal, lateral, and ventral arteries (see Prentiss and Arey, Fig. 274); a few of the larger ones will be noted. Follow the aorta posteriorly noting the ventro-lateral branches to the mesonephros and the dorsal segmental arteries to the neural tube. Just posterior to the stomach will be found a ventral artery running into the dorsal mesentery of the intestine; this is the future *coeliac artery* that supplies the stomach, intestine, etc. Smaller ventral arteries can be found, more posteriorly, both in the region of the small intestine (omphalomesenteric or superior mesenteric) and in the region of the large intestine (inferior mesen teric). Near the posterior level of the mesonephros, at the base of the hind limb, the two umbilical or allantoic arteries arise and continue on either side of the allantoic stalk, into the umbilical cord.[1] The dorsal aorta continues posteriorly as the median *caudal artery* (middle sacral artery of man).

Make a diagram of a lateral view of the arterial system of the pig, locating the following points by the number of the section in which they occur: (1) the union of the internal carotids to form the

[1] After birth the placental circulation is interrupted, the distal part of the umbilical arteries from the pelvis to the umbilicus (in man) atrophies and remains in the adult as the lateral umbilical ligaments on the anterior abdominal wall.

basilar artery, (2) base of the third aortic arches, (3) base of the sixth arches, (4) union of the two dorsal aortae, (5) origin of the umbilical arteries.

Venous system:

A. CHICK EMBRYO OF FORTY-EIGHT HOURS

At this stage two sets of veins are present: (1) a somatic, and (2) a splanchnic set. The former is composed, on each side, of an anterior cardinal and a posterior cardinal vein that unite to form the duct of Cuvier or common cardinal vein, which passes through the lateral mesocardium to open into the sinus venosus. Follow these vessels from the heart anteriorly and posteriorly. The *anterior cardinal*, at this time, runs between the wall of the otocyst and the myelencephalon and returns blood from the head regions; it will become the *internal jugular vein.* The *posterior cardinal* is closely associated in development with the mesonephros and disappears with the resorption of that organ at a much later stage.

The splanchnic system is that of the omphalomesenteric veins. Continuing from the sinus venosus posteriorly is a single median vessel, the *ductus venosus*, that is formed by a fusion of the omphalomesenteric veins. These continue on from the ductus venosus and pass by way of the diverging walls of the splanchnopleure to the yolk sac. Identify these veins through their entire extent.

B. CHICK EMBRYO OF SEVENTY-TWO HOURS

Associated with the cardinals at this time are two new veins, which are, however, still in a very rudimentary condition: (1) the *umbilical veins* will be found in the somatopleure posterior to the heart and opening into the ducts of Cuvier; and (2) the *subcardinal veins* are found immediately ventral to the mesonephros (see Lillie, Fig. 111). The umbilical veins become the allantoic veins in birds and in mammals they are the veins returning blood from the placenta. The subcardinals later play a large part in the formation of the vena cava inferior.

Little change appears in the vitelline veins with the exception that the ductus venosus (meatus venosus) is considerably elongated. Dorsal to this vessel is the anterior liver diverticulum while ventral to it and forming anastomoses around the sides is the posterior liver diverticulum. In this way the ductus venosus becomes surrounded by liver substance and becomes (except its proximal end) the portal vein.

Make a diagram of a lateral view of the venous circulation of the 72-hour chick and indicate the following points by numbers of sections: (1) anterior cardinal vein at point opposite otocyst, (2) ducts of Cuvier, (3) the bifurcation of the ductus venosus into the omphalo-mesenteric veins.

C. PIG EMBRYO OF 10-MM. LENGTH

The venous system of the 10-mm. pig may be divided into the following systems for study: (*a*) the cardinal veins—anterior, posterior, and ducts of Cuvier; (*b*) the vena cava inferior—anterior part of the ductus venosus, hepatic veins, subcardinal veins, and posterior portion of right postcardinal; (*c*) the umbilical veins; and (*d*) the portal system—posterior part of ductus venosus, and omphalomesenteric veins.

a) Identify the *anterior cardinal vein* (jugular) anterior to the heart and trace it to its origin in the head. Notice that it now lies external to the otocyst and cranial nerves. Since a small part of the sinus venosus is yet present, the openings of the *ducts of Cuvier* (common cardinals) into the heart are similar to the earlier stages.[1]

The *postcardinal veins* course posteriorly in the dorsolateral side of the mesonephros, and anastomoses are found quite freely with the subcardinals on the median surface between the aorta and the mesentery; they are interrupted about the middle

[1] The common cardinal of the left side (apparent right) is smaller than the right one, but can be traced into the remnant of the sinus venosus slightly posterior to the one on the right. Later an anastomosis is formed between the right and left anterior cardinals, anterior to the heart, and converts these into the innominate veins of the adult. The common cardinal (duct of Cuvier) on the left and part of the sinus venosus persist as the coronary sinus of the heart in the adult.

of their course by anastomoses with the subcardinals sufficiently large to direct most of the blood through these veins and thus into the inferior vena cava. Posteriorly the post-cardinals receive the *iliac vein* from the hind limb.

As development proceeds, the upper portion of the post cardinal veins atrophies for the most part, and the posterior portions are entirely changed (consult textbooks for fate of post cardinals).

b) *The vena cava inferior.*—In the 10-mm. pig a large part of the blood returning from the mesonephros (subcardinals), all from the intestine (portal) and that from the placenta (umbilical) enters the sinus venosus (right auricle) by a common vessel now known as the vena cava inferior (postcaval vein).

From the heart (sinus venosus, right auricle) trace the vena cava inferior, posteriorly, through the diaphragm and well into the liver. In the liver several large vessels and numerous liver sinusoids empty into it, making it almost impossible for the student to follow its channel posteriorly. It can easily be followed by identifying the vein posterior to the liver and tracing it into this organ to the point noted above.

In a section immediately posterior to the liver identify the *vena cava inferior* lying in the dorsal mesentery just ventral to the dorsal aorta (see Prentiss and Arey, Figs. 139, 140). From this

point trace the vein posteriorly to see that the subcardinals are separate veins. Follow the vena cava anteriorly from the point of identification. It passes ventrally into the liver on the right side into the large vessel previously followed from the heart. Since the subcardinal veins have a more direct route to the heart than do the posterior cardinal veins, blood from the posterior extremities formerly carried by the posterior cardinals is diverted into this newly formed channel (subcardinals and vena cava inferior) through the anastomoses, and as a consequence the posterior cardinals disappear at a somewhat later stage (consult textbooks for subsequent changes in vena cava inferior).

c) *The umbilical veins.*—In the 72-hour chick the umbilical veins were found to open into the ducts of Cuvier, but in the 10-mm. pig they have established a secondary connection through the liver with the ductus venosus. In a section through the umbilical cord identify the *umbilicals* lying in the somatopleuric walls. The left one (apparent right) is much larger than the right one, which very soon disappears. These veins return aerated blood from the placenta to the embryo. Trace them anteriorly into the liver (in some of the embryos the right vein will empty into the left one before the latter enters the liver). The left umbilical vein gives many small tributaries to the liver but

it is easily traced into the ductus venosus; blood from the ductus venosus flows into the vena cava inferior and thence into the heart.[1]

d) *The portal system.*—The student should understand the formation of the portal vein from the omphalo-mesenteric veins (consult textbooks for its previous history).

In the 10-mm. pig identify the portal vein in the walls of the gut at the level of the pancreas (see Prentiss and Arey, Fig. 140). From this point trace the vein posteriorly and note its division into the *vitelline vein* running out into the umbilical cord and the *superior mesenteric vein* that remains high up in the dorsal mesentery. From the pancreas, trace the vein anteriorly into the liver where it connects with the left umbilical vein; the vessel common to these two is the *ductus venosus*, which when traced anteriorly joins the vena cava inferior.

A considerable amount of blood carried by the portal vein as well as by the umbilical vein is diverted into the liver substance, the remainder entering the ductus venosus and then into the vena cava inferior: at birth when the umbilical ceases to function the ductus venosus atrophies posterior to its connection with the vena cava inferior. The result is that the blood from the

[1] After birth the umbilical vein no longer carries blood and its lumen disappears; it persists in the adult, from umbilicus to liver, as the round ligament of the liver (*ligamentum teres*) lying in the free edge of the falciform ligament.

intestine contained in the portal vein has to pass through the capillary system of the liver into the hepatic veins and thence through the postcava to the right auricle.[1]

Make a diagram of a lateral view of the venous system of the 10-mm. pig, locating by sections the following: (1) jugular vein opposite the otocyst, (2) point of entrance of anterior cardinal to the heart, (3) vena cava inferior passing through the diaphragm, (4) anastomosis of subcardinals.

Derivatives of Somites

The somites are derived from an aggregation of mesoderm of the segmental plate and differentiate from in front, posteriorly. Thus a series of primitive and more specialized somites is to be found in an embryo such as the 48-hour chick.

CHICK EMBRYO OF FORTY-EIGHT HOURS

Study a somite, the last or next to last one formed in your embryo. Note its outer epithelial layer of cells and the inner mesenchymal cells (see Lillie, Figs. 107, 110). Note the relations of somite and nephrotome. More anteriorly in the series the epithelial layer on the median face becomes interrupted by being converted into

[1] The embryonic connection between the portal vein and the vena cava inferior (part of the ductus venosus) that atrophies, persists in the adult as the ligamentum venosum Arantii in the substance of the liver.

mesenchyme that finally surrounds the notochord. This is known as the *sclerotome* and is the mesenchyme from which the cartilaginous mass and later bone of the vertebral column is derived. The notochord represents the central point about which ossification begins, or the central part of the centrum of a certebra.

The mesothelium from the sclerotome to the dorsal angle of the somite is known as the *myotome*, from which are derived the voluntary muscles (involuntary muscles are largely derivatives of mesenchyme). The peripheral or lateral face of the somite remains a thickened plate of cells and is known as the *dermatome*, the foundation of the dermis. For the further differentiation of these structures consult textbooks.

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